Intracerebral endotheliitis and microbleeds are neuropathological features of COVID-19

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Abstract: Coronavirus disease 19 (COVID-19), caused by infection with the severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2), has become a worldwide pandemic (1). Symptoms of COVID-19 vary widely and range from asymptomatic disease to severe pneumonia and multiorgan failure (2). A severe disease course is more likely in older patients and patients with pre-existing respiratory and cardiovascular conditions (2). Patients with severe Sars-CoV-2 infection may present with ischaemic stroke (3, 4) or even fatal intracerebral haemorrhage (5). To date, little is known about the neuropathological sequelae of COVID-19. The largest published autopsy series of COVID-19 neuropathology reported microthrombi and acute haemorrhagic infarction in a significant number of patients (6), while another more recent study found evidence of lymphocytic encephalitis and meningitis (7). Endotheliitis of the brain and extraneural organs has been shown in Sars-CoV infected patients (8). Similarly, it is a recurrent feature in the lungs and other peripheral organs of Sars-CoV-2 infected patients (9) but has not yet been reported in the central nervous system. We speculated that cerebrovascular pathology in COVID-19 patients could be a direct consequence of hitherto unidentified cerebral endotheliitis caused by Sars-CoV-2.

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Intracerebral endotheliitis and microbleeds are neuropathological features of COVID-19

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Main text

Coronavirus disease 19 (COVID-19), caused by infection with the severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2), has become a worldwide pandemic (1). Symptoms of COVID-19 vary widely and range from asymptomatic disease to severe pneumonia and multiorgan failure (2). A severe disease course is more likely in older patients and patients with pre-existing respiratory and cardiovascular conditions (2). Patients with severe Sars-CoV-2 infection may present with ischaemic stroke (3, 4) or even fatal intracerebral haemorrhage (5). To date, little is known about the neuropathological sequelae of COVID-19. The largest published autopsy series of COVID-19 neuropathology reported microthrombi and acute haemorrhagic infarction in a significant number of patients (6), while another more recent study found evidence of lymphocytic encephalitis and meningitis (7). Endotheliitis of the brain and extraneural organs has been shown in Sars-CoV infected patients (8). Similarly, it is a recurrent feature in the lungs and other peripheral organs of Sars-CoV-2 infected patients (9) but has not yet been reported in the central nervous system. We speculated that cerebrovascular pathology in COVID-19 patients could be a direct consequence of hitherto unidentified cerebral endotheliitis caused by Sars-CoV-2.

We retrospectively analysed all brain autopsies from Sars-CoV-2 infected patients referred to our department, for the presence of vasculopathic changes and cerebral haemorrhage (Supplementary Table 1). We excluded two patients with overt disseminated intravascular coagulopathy (10) (DIC, one patient was described previously (11)). Accordingly, we present a detailed neuropathological work-up of four Sars-CoV-2 infected patients.

Detailed clinical information is provided in Supplementary Table 1 and the Supplementary data. Four patients (3 males, 1 female, age range 70 - 81 years) presented with progressive respiratory symptoms, 3 were diagnosed with bilateral COVID-19 pneumonia during hospitalisation. Patient 2 also presented with bilateral pneumonia, consistent with COVID-19, and the lung specimen taken at autopsy tested positive for Sars-CoV-2,

although two previous nasopharyngeal swabs were negative. None of the patients showed laboratory signs of overt DIC (Supplementary Table 1) (10). *Ante-mortem* cranial tomography studies performed in patient 1 were unremarkable. All patients died 5-15 days after admission.

Endotheliitis in the lungs of patient 1 (9) and inflammatory olfactory neuropathy in patients 1 and 3 have been described earlier (12). Gross examination of the brains was unremarkable except in two cases (patients 1 and 4), which revealed diffuse petechial haemorrhage, most prominent at the grey-white matter junction of the neocortex (Figure 1a, Supplementary Table 1). Corresponding *post-mortem* magnetic resonance imaging (MRI) of one brain showed multiple cerebral petechial haemorrhages on susceptibility weighted imaging (SWI) (patient 1, Figure 1b). On histology, the vast majority of the haemorrhages were fresh (Figure 1c), without evidence of haemoglobin breakdown products on Prussian blue stains. Juxtacortical microbleeds were observed in all patients, most conspicuous in the frontal lobe. Additionally, petechial haemorrhages were observed in the thalamus, mesencephalon and pons (Supplementary Table 1). In one case, multiple intraparenchymal subacute haemorrhages were found in the corpus callosum (patient 4, Figure 1d). Fresh haemorrhages were both perivascular, as well as intraparenchymal without relation to vasculature. In all cases, there was evidence of diffuse intravascular thrombosis. In small veins of the basal ganglia of two patients (1 and 3), intra-endothelial lymphocytic and monocytic inflammation with occasional apoptotic figures were observed, consistent with the previously reported “endotheliitis” in Sars-CoV-2 infected patients (9) (patients 1 and 3, Figure 1e). Immunohistochemistry for ACE2 consistently revealed detectable expression in all bar one patient diagnosed with COVID-19 (Supplementary Figure 1). Additional staining of three pre-pandemic autopsy controls revealed very faint or absent ACE2 expression (Supplementary Figure 1 and Supplementary Data). To test whether the younger age of the controls confounded ACE2 expression, we compared ACE2 transcripts from basal ganglia from the Genotype-Tissue Expression project (GTEx, total of n = 205 patients). We did not see increased gene expression during aging (Supplementary Data). Additional lymphocytic “cuffing” was observable in two cases (patients 1 and 3). Congo red stains and immunohistochemistry for beta-amyloid were negative in all cases.

Cerebral petechial haemorrhages may represent a histological correlate of the neurological symptoms observed in the COVID-19 patients described in this case series. Endothelial cell infection is a recurrent feature in the lungs and other peripheral organs of COVID-19 patients (9) but has not yet been reported in the brain (6, 7). We report here for the first time the presence of intracerebral endotheliitis in two patients diagnosed with Sars-CoV-2 infection. The observed endotheliitis could be an autoimmune, late-onset phenomenon or a direct effect of endothelial infection as angiotensin-converting enzyme 2 (ACE2), the Sars-CoV-2 receptor, is expressed in the brain vasculature (Supplementary Figure 1 and (13)). Here, we found higher ACE2 expression in the brain vasculature of patients with endotheliitis than in COVID-19 patients without endotheliitis or than in control patients (Supplementary Figure 1). Although control patients were younger on average, comparison of publicly available gene expression data did not show increased ACE2 expression in the basal ganglia during aging (Supplementary Data). The small number of patients analysed, however, precludes a causal inference.

Recently, the concept of critical illness-associated microbleeds (CRAM) was introduced (14). The topology of the microbleeds described in this condition is somewhat similar, but not identical, to the patients in our cohort as well as in hypoxaemic patients after high-altitude exposure (14). Correspondingly, hypoxia was the cause of death in all patients from the present case series. The nosological distinction between microbleeds in critically ill patients and COVID-19 patients is not entirely clear. CRAM show some predilection for the corpus callosum, while in our case series, this pattern was only observed in one patient. Similarly, 3 out of 4 patients in our series showed a marked involvement of the brainstem, in contrast to 4 out of 25 patients described by Fanou *et al.* (14) Still, haemorrhages might have been aggravated by concomitant acute respiratory distress syndrome.

All patients suffered from arterial hypertension and hypertensive microangiopathy, however, hypertensive microbleeds favour the deep grey matter and the infratentorial region (15). Elevated risk for thrombosis and pulmonary embolism is well-document in COVID-19 patients, and all the patients received prophylactic anticoagulants and/or

antiplatelet therapy, which may have predisposed them to haemorrhagic events. Hydroxychloroquine, which was administered to three patients, has also been reported to cause bleeding (16). On autopsy, no haemorrhage was seen at predilection sites such as the gastrointestinal tract, thus making solely drug-induced haemorrhage unlikely. (16)

In both patients suffering from prolonged coma and negative wake-up attempts, intraparenchymal haemorrhages had already been observed grossly suggesting a positive correlation between the severity of the vasculopathy and acute sleep-wake dysregulation. Accordingly, the distribution of microbleeds showed a neuroanatomical preference for central modulators of wakefulness (pons, mesencephalon and paramedian thalamus, Figure 1e). A similar distribution of brainstem lesions leading to disturbed sleep-wake regulation can be observed in neurodegenerative diseases, traumatic brain injury and acute vascular events (17). A major drawback of our study is the plethora of co-morbidities, such as acute respiratory distress syndrome, hypertension and prophylactic anticoagulation, all of which increase the risk of intracerebral haemorrhage. It seems likely that we investigated an at-risk cohort, which may explain the unusually high occurrence of intracerebral bleeding in our case series compared to another recently published study (18). Although it is tempting to deduce a causal connection between intracerebral haemorrhage, Sars-CoV-2 induced endothelial inflammation and hypoxaemic damage, the retrospective nature of this study and the small number of patients allows for limited conclusions and necessitates further studies.

Neurological symptoms associated with COVID-19 have been described with manifold aetiologies, such as ischaemic stroke, haemorrhagic encephalopathy and others (19). In contrast to a recently published case report, we did not observe signs of perivascular demyelination (20). Cerebral microbleeds have been associated with increased risk for cardiovascular mortality (21) and cognitive deterioration (22). Additionally, emerging evidence suggests rapidly waning humoral anti-Sars-CoV-2 immunity might be associated with a risk of recurrent infection and subsequent cognitive dysfunction (23). The temporal evolution of COVID-19-associated cerebrovascular pathology remains unclear. Future studies could clarify whether endothelial inflammation is self-limiting or if

similar pathological changes can be observed in COVID-19 patients without neurological symptoms.

Figure legend

Figure 1. (A) Gross examination was significant for multiple, mostly juxtacortical haemorrhages (patient 1). (B) Post-mortem susceptibility weighted imaging (SWI) of brain revealed multiple microbleeds/haemorrhages (patient 1). (C) H&E-stained section showing fresh haemorrhages in the centrum semiovale (patient 2). (D) Subacute haemorrhage containing macrophages (CD68, right insert) and blood breakdown products (Prussian blue, Fe, left insert) in the corpus callosum of patient 4. (E) Diffuse intravascular microthrombosis and endotheliitis in the basal ganglia of patient 1. Elevated apoptosis, as demonstrated by cleaved caspase 3 immunohistochemistry, was observable in endothelial cells and intramural infiltrates but not in the adjacent parenchyma (cleaved casp. 3, left insert). Intra-endothelial lymphocytes stained positive on CD45 immunohistochemistry (CD45, right insert). (F) Schematic localisation of the intracerebral haemorrhages: 1) frontal cortex 2) other isocortical areas, as well as deep grey matter 3) mesencephalon 4) pons 5) medulla oblongata.

Ethical statement

Informed consent for autopsy and publication was given by next-of-kin in all cases. Case series do not need institutional review board approval according to Swiss legislation.

Data sharing

ACE2 stained slides and the detailed analysis pipeline for ACE2 gene expression comparison across age groups in R3.6.3 are made publicly available via Figshare doi: 10.6084/m9.figshare.13089383. All other pathological slides described in this manuscript are publicly available via Synapse (synapse ID: syn23532584). Pathological slides can be analysed with common open-source software such as QuPath https://qupath.github.io.

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Table 1: Clinical and pathological characteristics of the patients

Variable	Patient 1	Patient 2	Patient 3	Patient 4
Age, y	70	77	79	81
Gender	Male	Female	Male	Male
Pre-existing disease; Cardiovascular risk factors	Hypertension, coronary artery disease, atrial fibrillation, kidney transplantation 2013	Hypertension, depression	Obesity, paroxysmal atrial fibrillation, severe pulmonary hypertension, chronic renal failure, M. Waldenström	Hypertension, coronary artery disease, chronic renal insufficiency
Pre-existing disease; medication	ASA, Tacrolimus, Mycophenolat, Bisoprolol, Amlodipin	Lithium sulfate, Bisoprolol	Verapamil, Tadalafil, Macitentan, Apixaban, Toresamid, Eplerenon	ASA, Losartan, Metolprolol, Ranolazin, Ezetimib. Rosuvastin
Clinical course				
Empirical COVID-19 treatment	Hydroxychloroquine	None	Hydroxychloroquine	Hydroxychloroquine, Remdesivir
Pre-intubation/ worst S_PO_2	89%	No intubation / 53% measured 2 days before death	No intubation / 70%	89%
Anticoagulation in the last 3 days before CNS disorder	ASA at admission, therapeutic anticoagulation with unfractionated heparin in ICU	Prophylactic Enoxaparine 40 mg/d	Apixaban	Prophylactic dosage with unfractionated heparin
CNS disorder	Confusion at admission, asymmetric reactive pupils, negative wake-up	No CNS disorder	No CNS disorder	Negative wake-up
Concomitant condition*	Invasive mechanical ventilation,	O_2-Therapy via high flow	O_2-Therapy	Invasive mechanical ventilation,

	prone positioning CRRT, mesenteric ischaemia, myocardial injury	mask/nasal cannula		pneumothorax
Relevant pathological laboratory values at time of CNS disorder	CRP 299 mg/l, lymphocyte count 0,29 G/l, IL-6 3919 ng/l, fibrinogen 6,7 g/l, d-dimer 2.42 mg/l, thrombocyte count 177 G/l	CRP 145 mg/l, lymphocyte count 0.78 G/l, thrombocyte count 427 G/l, fibrinogen 8.79 g/l	CRP 246 mg/l, lymphocyte count 0,52 G/l, IL-6 NA , fibrinogen NA, d-dimer NA, thrombocyte count 238 G/l	CRP 227 mg/l, lymphocyte count 0,82 G/l, IL-6 175 ng/l, fibrinogen 5,9 g/l, d-dimer 11,1 mg/l
DIC score **	3	NA	NA	3
Time from disease onset to manifestations of CNS disorder, days	2	No CNS disorder	No CNS disorder	14
Neuroimaging	CT Scan: unremarkable Post-mortem brain MRI / SWI-sequence: Multiple microbleeds/haemorrhages	NA	NA	NA
Outcome at ICU discharge	Died from multi-organ failure and mesenterial ischaemia	Died under palliative care	Died from hypoxaemia and severe pulmonary hypertension under palliative care	Died from cardio-pulmonary failure under palliative care
Pathological findings				
Brain weight, g	1374	1239	1364	1626
Gross pathology	Diffuse oedema; punctuate haemorrhage (frontal and parietal cortex, basal ganglia, pons, corpus callosum)	unremarkable	unremarkable	Mild, patchy atherosclerosis; mild diffuse atrophy; punctuate haemorrhage (corpus callosum, hypothalamus, frontal and temporal cortex)

Haemorrhages				
Neocortex	+	+	+	+***
Hippocampus	-	+	-	-
Basal ganglia	-	+	+	-
Thalamus / Hypothalamus	-	+	+	+
Stria olfactoria	+	+	-	-
Mesencephalon	+	+	+	-
Pons	+	+	+	-
Medulla oblongata	+	+	-	-
Cerebellum	-	+	-	-
Other microscopic findings	Endotheliitis, diffuse intravascular thrombosis, perivasal lymphocytic infiltrates, hypertensive microangiopathic changes, calcifications dentate gyrus	Intravascular microthrombi, hypertensive microangiopathic changes	Endotheliitis, a neuron with granulovacuolar degeneration in the hippocampus, hypertensive microangiopathic changes	Brainstem-predominant alpha synucleinopathy, hypertensive microangiopathic changes, subacute ischaemia frontal cortex
Cerebral amyloid angiopathy (Congo red / beta amyloid)	-	-	-	-

ASA acetylsalicylic acid, CNS central nervous system, CRP C-reactive protein, CRRT continuous renal replacement therapy, CT computed tomography, DIC disseminated intravascular coagulopathy, ICU intensive care unit, IL-6 Interleukine 6, MRI magnetic resonance imaging, NA not available, SpO_2 peripheral oxygen saturation, SWI Susceptibility weighted imaging,

* Concomitant condition: additional findings which occurred at the time of the neurological deficits

** DIC score according to International Society on Thrombosis and Haemostasis [11]

*** this patient harboured fresh juxtacortical haemorrhages and multiple subacute haemorrhages in the corpus callosum